JSBS Journal of Sistan and Baluchistan Studies
Volume 5, Issue 1 (2025) 49-57
Doi: 10.22034/jsbs.2025.515626.1082

TRACING THE ROOTS OF PLANT DISEASES: ARCHAEOLOGY, PLANT PALEOPATHOLOGY, AND THE HISTORY OF PLANT-PATHOGEN INTERACTIONS

Mojtaba KEYKHASABER[1]✉

[1] Associate Professor, Department of Plant Pathology, University of Zabol, Zabol, Iran, (mkeikhasaber@uoz.ac.ir)

Received: 09 February 2025 Accepted: 18 April 2025 Available online: 20 April 2025

Abstract: The study of plant diseases in archaeological contexts, known as plant paleopathology, offers critical insights into the historical interactions between plants, pathogens, and human societies. Plant diseases have significantly influenced agricultural practices, economic systems, and even the fate of civilizations, as seen in the Irish Potato Famine (caused by Phytophthora infestans) and the rust epidemics that impacted the Roman Empire. By analyzing archaeological plant remains, researchers reconstruct the history of plant pathogens, assess their societal impacts, and derive lessons for modern agriculture. This review explores the methods used to detect plant pathogens in ancient materials, including morphological analysis, ancient DNA (aDNA) sequencing, proteomics, spectroscopy, immunological assays, stable isotope analysis, and paleohistology. These techniques have enabled the identification of pathogens such as Claviceps purpurea (ergot), Puccinia graminis (wheat rust), and Phylloxera vitifoliae (grapevine pest), revealing their evolutionary and ecological histories. Case studies, such as the Irish Potato Famine and the 19th-century coffee rust epidemic in Ceylon, demonstrate how plant diseases have driven agricultural transformations, scientific advancements, and policy changes. The integration of archaeological evidence with modern biomolecular techniques provides a multidisciplinary framework for understanding long-term plant-pathogen dynamics. Future research should leverage advances in high-throughput sequencing, proteomics, and computational modeling to further explore ancient disease outbreaks, pathogen evolution, and human adaptation strategies. By bridging archaeology and plant pathology, this field enhances our ability to address contemporary challenges in food security, crop resilience, and sustainable agriculture.

Keywords: Plant paleopathology, ancient DNA, archaeobotany, crop diseases, pathogen evolution, agricultural history.

چکیده: مطالعه بیماری‌های گیاهی در زمینه‌های باستان‌شناسی، که به عنوان دیرین‌آسیب‌شناسی گیاهی شناخته می‌شود، بینش‌های مهمی در مورد تعاملات تاریخی بین گیاهان، عوامل بیماری‌زا و جوامع انسانی ارائه می‌دهد. بیماری‌های گیاهی تأثیر قابل توجهی بر شیوه‌های کشاورزی، سیستم‌های اقتصادی و حتی سرنوشت تمدن‌ها داشته‌اند، همان‌طور که در قحطی سیب‌زمینی ایرلند (ناشی از Phytophthora infestans) و همه‌گیری زنگ‌ها که امپراتوری روم را تحت تأثیر قرار داد، مشاهده می‌شود. با تجزیه و تحلیل بقایای گیاهی باستان‌شناسی، محققان تاریخچه عوامل بیماری‌زای گیاهی را بازسازی می‌کنند، تأثیرات اجتماعی آن‌ها را ارزیابی می‌کنند و درس‌هایی برای کشاورزی مدرن استخراج می‌کنند. این مرور به بررسی روش‌های مورد استفاده برای تشخیص عوامل بیماری‌زای گیاهی در مواد باستانی می‌پردازد، از جمله تحلیل ریخت‌شناسی، توالی‌یابی DNA باستانی (aDNA)، پروتئومیک‌ها، طیف‌سنجی، آزمون‌های ایمونولوژیکی، تحلیل ایزوتوپ‌های پایدار و دیرین‌بافت‌شناسی. این تکنیک‌ها شناسایی عوامل بیماری‌زایی مانند Claviceps purpurea (ارگوت)، Puccinia graminis (زنگ گندم) و Phylloxera vitifoliae (آفت مو) را ممکن ساخته و تاریخچه تکاملی و اکولوژیکی آن‌ها را آشکار کرده‌اند. مطالعات موردی، مانند قحطی سیب‌زمینی ایرلند و همه‌گیری زنگ قهوه در سیلان در قرن نوزدهم، نشان می‌دهند که چگونه بیماری‌های گیاهی منجر به دگرگونی‌های کشاورزی، پیشرفت‌های علمی و تغییرات سیاستی شده‌اند. ادغام شواهد باستان‌شناسی با تکنیک‌های زیست‌مولکولی مدرن، چارچوبی چندرشته‌ای برای درک پویایی‌های بلندمدت گیاه ـ عامل بیماری‌زا فراهم می‌کند. تحقیقات آینده باید از پیشرفت‌ها در توالی‌یابی با توان بالا، پروتئومیک‌ها و مدل‌سازی محاسباتی بهره ببرد تا به بررسی بیشتر شیوع بیماری‌های باستانی، تکامل عوامل بیماری‌زا و راهبردهای انطباق انسانی بپردازد. با پیوند باستان‌شناسی و آسیب‌شناسی گیاهی، این حوزه توانایی ما را برای مقابله با چالش‌های معاصر در امنیت غذایی، مقاومت محصولات و کشاورزی پایدار افزایش می‌دهد.

کلیدواژه: دیرین‌آسیب‌شناسی گیاهی، DNA باستانی، باستان‌گیاه‌شناسی، بیماری‌های محصولات کشاورزی، تکامل عوامل بیماری‌زا، تاریخ کشاورزی.

I. Introduction

The study of plant diseases in archaeological contexts, often referred to as plant paleopathology, provides a unique window into the interactions between plants, pathogens, and human societies throughout history (Labandeira & Prevec, 2014). Plant diseases have shaped agricultural practices, influenced economic systems, and even altered the course of civilizations, such as the impact of plant diseases on the Roman empire which had to deal with a rust disease (cause by a fungal agent) (Iriti & Faoro, 2008), and the emigration of many Irish to America as a result of the disease called "late-blight" (causes by *Phytophthora infestans*) that destroyed the potato crop and caused a great famine in Ireland (Reader, 2009).

By examining archaeological plant remains, researchers can reconstruct the history of plant pathogens, understand their impact on ancient societies, and draw lessons for modern agriculture (Agrios, 2005; Zohary et al., 2012; McNeill, 1976; Martin et al., 2013). It is also closely linked to broader themes in environmental archaeology and historical ecology. For example, the spread of crop diseases has often been associated with the intensification of agriculture, the movement of crops and people, and climatic changes (Fuller & Lucas, 2017). By examining the archaeological

record of plant diseases, researchers can gain insights into how past societies adapted to agricultural challenges, such as crop failure and famine, and how these events influenced cultural and technological innovations (Zhou & Li, 2019).

One of the key challenges in the archaeology of plant diseases is the identification and interpretation of disease-related evidence. Plant pathogens can leave subtle traces in the archaeological record, such as deformities in seeds, fungal spores, or molecular signatures of infection. Advances in biomolecular techniques, particularly the analysis of aDNA, have revolutionized the field by enabling the detection of pathogens in ancient plant remains (Ristaino et al., 2001; Martin et al., 2013). These methods have been instrumental in studying high-profile historical events, such as the Irish Potato Famine, and have provided new insights into the origins and evolution of plant diseases (Yoshida et al., 2013).

The archaeology of plant diseases also contributes to our understanding of long-term human-environment interactions. For instance, the introduction of new crops and agricultural practices often led to the emergence of new disease pressures, which in turn influenced settlement patterns, trade networks, and social organization (Redman, 1999). By studying the archaeological evidence of plant diseases, researchers can explore how past societies managed these challenges and how their experiences might inform modern efforts to address food security and sustainable agriculture (McNeill, 2012).

This review explores the archaeology of plant diseases, focusing on the methods used to detect pathogens in historical materials, the insights gained from these studies, and the opportunities for future research. By integrating archaeological evidence with modern scientific techniques, this field offers a multidisciplinary approach to understanding the co-evolution of plants, pathogens, and humans.

II. Historical and Archaeological Evidence of Plant Diseases

II1. Ancient Civilizations and Early Records

Plant diseases have been documented since the dawn of agriculture. Ancient texts, such as the Ebers Papyrus (1550 BCE) from Egypt, mention crop diseases and their effects on staple foods like wheat and barley (Large, 1940). Similarly, Greek and Roman agricultural writers, including Theophrastus and Pliny the Elder, described symptoms of plant diseases, though their understanding of the causes was limited (Zohary et al., 2012). Archaeological evidence from ancient sites supports these textual records. For example, charred grains and seeds from Neolithic and Bronze Age settlements often show signs of fungal infections, such as ergot (*Claviceps purpurea*), which affected cereals like rye and barley (Carefoot & Sprott, 1967). These findings highlight the long-standing relationship between humans and plant pathogens.

II2. Medieval and Renaissance Periods

During the Middle Ages, monastic records and agricultural manuals documented crop failures and diseases, often attributing them to environmental factors or divine punishment (Large, 1940). The Renaissance saw the emergence of botanical illustrations, which depicted diseased plants and their symptoms. These records provide valuable insights into the prevalence and impact of plant diseases during these periods. Archaeological studies of medieval plant remains have identified pathogens such as *Puccinia graminis* (wheat stem rust) and *Ustilago* spp. (smut fungi) in grain stores and agricultural sites (Zohary et al., 2012). These findings suggest that plant diseases were a significant concern for medieval farmers.

II3. Major Historical Outbreaks

Several historical plant disease outbreaks have profoundly impacted human societies, shaping agricultural policies, driving scientific advancements, and influencing global crop management practices.. The Irish Potato Famine (1845–1852), caused by *Phytophthora infestans*, is one of the most well-documented examples. Ireland's overreliance on a single potato variety (*Lumper*) led to catastrophic famine. Post-famine, governments promoted crop diversification to reduce vulnerability (Bourke, 1993). Moreover, The British government's laissez-faire policies were heavily criticized, leading to poor laws and eventual land reforms in Ireland (Kinealy, 1994). The outbreak also spurred early biosecurity measures to prevent pathogen spread (Ristaino, 2001). The famine was a catalyst for mycology as a scientific discipline. Anton de Bary (1861) identified *P. infestans* as the cause, laying the foundation for plant pathology (Large, 1940). In addition, Modern potato breeding programs (e.g., by the International Potato Center) trace their origins to post-famine efforts (Reader, 2008). Also, genetic analysis of 19th-century potato samples revealed pathogen evolution, helping predict future outbreaks (Yoshida et al., 2013; Martin et al., 2013; Ristaino et al., 2001).

Another notable example is the coffee rust epidemic (*Hemileia vastatrix*) in Ceylon (now Sri Lanka) during the late 19th century, which devastated coffee plantations and led to a shift to tea cultivation (Carefoot & Sprott, 1967). Indeed, the British colonial government abandoned coffee in favor of tea plantations, transforming Sri Lanka into a leading tea producer (McCook, 2019). Additionally, the collapse of Ceylon's coffee industry shifted production to Latin America and Africa (Avelino et al., 2015). The epidemic led to

stricter plant import/export controls to prevent cross-continental pathogen spread (Carefoot & Sprott, 1967). In terms of the impact on scientific advancements, the crisis accelerated research into copper-based fungicides (e.g., Bordeaux mixture), later used worldwide (Large, 1940), scientists began studying rust-resistant coffee varieties, influencing modern breeding (Talhinhas et al., 2017), and the outbreak contributed to early disease spread models in tropical agriculture (McCook, 2006).

II4. Insights from the Archaeology of Plant Diseases

Archaeological studies have provided valuable insights into the evolution of plant pathogens. For example, genetic analysis of *Phytophthora infestans* from historical potato samples has revealed changes in the pathogen's genome associated with increased virulence and host adaptation (Martin et al., 2013). Moreover, archaeological evidence has helped trace the geographic spread of plant diseases. For instance, the introduction of *Phylloxera vitifoliae* to Europe in the 19th century, which devastated grapevine populations, has been documented through historical records and archaeological plant remains (Carefoot & Sprott, 1967). In addition to the valuable information about evolution and the spread of plant pathogens, the archaeology of plant diseases highlights the profound impact of pathogens on human societies. Crop failures caused by plant diseases have led to famine, economic collapse, and social upheaval. For example, the Irish Potato Famine resulted in mass starvation, emigration, and significant demographic changes (McNeill, 1976).

III. Plant Pathogens Detection Methods in Archaeological Materials

Detecting plant pathogens in ancient plant debris is a fascinating and multidisciplinary endeavor that integrates archaeology, plant pathology, molecular biology, paleobotany, and microbial genomics to uncover the hidden history of plant diseases and their impacts on past ecosystems. By analyzing preserved plant remains—such as seeds, leaves, and wood fragments—from archaeological sites, researchers can reconstruct ancient disease outbreaks, track pathogen evolution, and understand how plant-pathogen interactions shaped early agriculture and human societies. This approach relies on advanced DNA sequencing, microscopy, and biochemical techniques to identify microbial signatures in degraded plant material, while also confronting challenges such as biomolecule degradation, contamination risks, and the scarcity of well-preserved samples. Ultimately, studying ancient plant pathogens not only sheds light on historical agricultural crises (like the Irish Potato Famine) but also informs modern strategies for crop resilience, disease surveillance, and biosecurity in a changing climate.. Below are the primary methods used for detecting plant pathogens in ancient plant materials, along with their applications, advantages, and limitations.

IV. Morphological Analysis

Morphological analysis is one of the oldest and most straightforward methods for identifying plant pathogens in ancient plant materials. This approach involves the visual and microscopic examination of plant remains to detect physical characteristics of pathogens and their effects on plant tissues, such as fungal spores, hyphae, sclerotia, nematode cysts, or insect damage, making it a valuable tool in archaeobotany and plant paleopathology. For example, the presence of ergot sclerotia in ancient grain stores from Neolithic and Bronze Age sites provides direct evidence of fungal infections caused by *Claviceps purpurea* (Zohary et al., 2012; Van der Veen, 2007). These structures are easily recognizable due to their dark, elongated shape and are often found in cereal grains like rye and barley (Zohary et al., 2012). Also, fungal pathogens like *Puccinia graminis* (wheat stem rust) and *Ustilago* spp. (smut fungi) leave distinctive spores and hyphae that can be identified under a microscope (Carefoot & Sprott, 1967).

Insects, such as gall-forming nematodes or wasp, can induce abnormal growths (galls) in plant tissues, which are often preserved in archaeological remains. These galls serve as direct evidence of insect-plant interactions in ancient ecosystems. Additionally, insect feeding marks on seeds, leaves, or stems provide valuable insights into pest activity in ancient agricultural systems (Panagiotakopulu, 2001). For example, the presence of characteristic feeding patterns on seeds can indicate infestations by beetles or other pests (Van der Veen, 2007; Kenward & Hall, 1995). Furthermore, nematode cysts or egg masses in root tissues can be identified using microscopy, offering evidence of nematode infections in ancient crops. These structures are often preserved in waterlogged or charred archaeological contexts, allowing researchers to study the impact of nematodes on ancient agriculture (Cappers & Neef, 2012; Pearsall, 2015).

Morphological analysis offers several advantages in the study of ancient plant pathogens. First, it is non-destructive, allowing researchers to preserve the integrity of valuable archaeological samples. Second, it is cost-effective, requiring minimal equipment compared to advanced molecular techniques. Third, it enables the immediate identification of pathogen structures, such as fungal spores, nematode cysts, or insect damage, without the need for complex laboratory procedures (Pearsall, 2015; Cappers & Neef, 2012). Despite its advantages, morphological analysis has

several limitations. It is restricted to visible structures, meaning only pathogens that leave distinct physical traces can be detected. Many pathogens, particularly bacteria and viruses, do not produce distinctive morphological features, making them difficult to identify using this method. Additionally, it is often challenging to distinguish between closely related species or strains based on morphology alone. Furthermore, ancient plant materials are frequently degraded, which can obscure or destroy pathogen structures, complicating their identification (Van der Veen, 2007; Zohary et al., 2012). To overcome these limitations, morphological analysis is often combined with other techniques. For example, scanning electron microscopy (SEM) enhances the resolution of microscopic structures, allowing for more detailed observations, molecular techniques, and stable isotope analysis (Warinner et al., 2017; Cappellini et al., 2018).

V. Ancient DNA (aDNA) Analysis

The study of aDNA, often referred to as paleogenomics or metagenomics when applied to environmental samples, has transformed our understanding of evolutionary history. ADNA analysis involves, isolating DNA from ancient plant debris or pathogen structures (e.g., fungal spores, bacterial cells), amplification using polymerase chain reaction (PCR) to amplify specific DNA regions of interest, sequencing and determining the nucleotide sequence of the amplified DNA to identify the pathogen, and finally bioinformatics analysing the sequence data to compare it with modern pathogen genomes and reconstruct evolutionary relationships. By extracting and analyzing genetic material from ancient remains, researchers can reconstruct the genomes of long-extinct species and pathogens, shedding light on their biology, evolution, and interactions with their environments. This approach has provided unprecedented insights into the genetic diversity of ancient populations, the origins of diseases, and the evolutionary processes that have shaped life on Earth (Shapiro & Hofreiter, 2014; Orlando, et al., 2021; Smith, et al. 2014; Wales, et al. 2016). Among these, ancient plant pathogens have garnered significant attention due to their profound impact on agriculture and ecosystems (Martin & Cappellini, 2015). ADNA analysis method has been instrumental in studying historical outbreaks of plant diseases, such as the Irish Potato Famine caused by *Phytophthora infestans*, in which aDNA analysis of 19th-century potato samples has confirmed the presence of *P. infestans* and provided insights into its genetic diversity (Martin, et al., 2013; Martin & Kamoun, 2012; Ristaino, et al., 2001). Also, aDNA analysis has identified ergot (*Claviceps purpurea*) DNA in ancient grain stores, providing insights into the historical prevalence of ergotism (Zohary, et al., 2012). The historical spread of bacteria *Xylella fastidiosa*, which causes diseases in crops like grapes and olives, was likewise studied using aDNA analysis (Nunney, et al. 2014; Almeida, & Nunney, 2015). Identification of tobacco mosaic virus (TMV) RNA in ancient tobacco leaves, and the agent of downy mildew (*Plasmopara viticola*) in grapes were another examples of aDNA analysis application for studying the plant pathogens in archaeological contexts (Rouxel, et al. 2013; Gobbin, et al. 2006). The first reconstruction of an ancient RNA genome from archaeological barley grain helpt to identify a 750-year-old strain of Barley Stripe Mosaic Virus (BSMV). Phylogenetic analysis reveals that BSMV likely originated around 2000 years ago in the Near East or North Africa, spreading globally via historical trade routes. The inclusion of ancient viral data corrected previous estimates of a recent origin, highlighting the importance of archaeological evidence in understanding viral evolution and plant disease history (Smith, et al. 2014). Also, analyzes of genomic methylation patterns in archaeological barley grains from Egypt (800 BCE–1812 CE), revealed that 5-methylcytosine de-methylation occurs as a time-dependent diagenetic process with an estimated half-life of ~713 years. A virus-infected sample showed elevated methylation, supporting the role of stress in epigenetic changes, while fragmentation and chemical deamination contributed to methylation loss over time. The findings highlight the potential of ancient DNA methylation studies to uncover past environmental stress responses and epigenetic decay dynamics (Smith, et al. 2014).

In spite of the advantages of aDNA analysis including (high specificity (Bos, et al. 2011; Paabo, et al. 2004), evolutionary insights, sensitvity (Marciniak & Poinar, 2018; Shapiro & Hofreiter, 2014), and complementary to other methods (Cappellini, et al. 2018; Warinner, et al. 2017), this method has challenges and limitations including; DNA degradation; aDNA is often fragmented and degraded, making extraction and amplification challenging (Allentoft, et al. 2012; Briggs, et al. 2007), contamination; risk of contamination from modern DNA during sampling or laboratory procedures (Cooper, & Poinar, 2000; Gilbert, et al. 2005), limited reference genomes; lack of reference genomes for ancient pathogens can complicate data analysis (Der Sarkissian, et al. 2015; Orlando, et al. 2015), and cost and expertise; requires specialized equipment, reagents, and expertise in molecular biology and bioinformatics (Hofreiter, et al. 2001; Knapp & Hofreiter, 2010). Advances in high-throughput sequencing technologies, particularly next-generation sequencing (NGS), have significantly expanded the scope of ancient DNA (aDNA) studies. These technologies enable the reconstruction of ancient pathogen genomes with unprecedented resolution, providing detailed insights into their genetic diversity, evolution, and historical spread (Knapp & Hofreiter,

2010; Shapiro & Hofreiter, 2014). Additionally, improvements in DNA extraction and purification techniques have enhanced the recovery of aDNA from highly degraded and low-concentration samples, making it possible to analyze even the most challenging ancient materials (Allentoft et al., 2012; Orlando et al., 2021). The development of paleogenomic databases is another critical advancement, facilitating the comparison of ancient and modern pathogen genomes. These databases provide a valuable resource for identifying genetic changes over time and understanding the evolutionary dynamics of plant pathogens (Der Sarkissian et al., 2015; Marciniak & Poinar, 2018). Furthermore, interdisciplinary collaboration between archaeologists, plant pathologists, and molecular biologists is essential for unlocking new insights into the history of plant diseases. By combining expertise from these fields, researchers can integrate aDNA analysis with archaeological, morphological, and environmental data to build a more comprehensive understanding of ancient plant-pathogen interactions (Warinner et al., 2017; Cappellini et al., 2018).

VI. Proteomics

Proteomics is defined as the large-scale analysis of proteins, encompassing their structures, functions, and interactions within biological systems (Wilkins et al., 1996). In the study of ancient plant remains, proteomics is particularly focused on the identification and characterization of proteins derived from both plants and their associated pathogens (Shevchenko et al., 2001). Proteins often exhibit greater stability compared to DNA in archaeological contexts, which makes proteomic approaches particularly advantageous for investigating historical plant diseases (Cappellini et al., 2014). This analytical method provides critical insights into pathogen activity, the defensive responses of host plants, and the underlying biochemical mechanisms governing plant-pathogen interactions (Rasmussen et al., 2012). By leveraging proteomics, researchers can reconstruct historical disease dynamics and better understand the co-evolution of plants and pathogens over time (Warinner et al., 2014). This method has widely been employed to investigate plant pathogens across various archaeological and historical contexts. For instance, studies have identified fungal pathogens (Cappellini et al., 2014; Zohary et al., 2012) and bacterial pathogens (Nunney et al., 2014) in ancient plant materials.

Proteomics involves a series of systematic steps to analyze proteins from complex biological samples. The process begins with sample preparation, where ancient plant debris is meticulously collected and prepared for protein extraction to minimize contamination and degradation (Cappellini et al., 2014; Rakhshani & Mansouri-Birjandi, 2018). Next, protein extraction is performed using chemical or mechanical methods to isolate proteins from the sample matrix (Shevchenko et al., 2001). Following extraction, separation techniques such as gel electrophoresis or liquid chromatography are employed to fractionate the protein mixture based on properties like size, charge, or hydrophobicity (Aebersold & Mann, 2003). Subsequently, identification is carried out using mass spectrometry (MS), which determines proteins based on their mass-to-charge ratio and fragmentation patterns (Yates et al., 2009). Finally, bioinformatics analysis is conducted, where computational tools are utilized to process the MS data, identify proteins by matching them against reference databases, and interpret their biological significance (Nesvizhskii, 2014). This integrated approach enables researchers to uncover insights into protein composition, function, and interactions in ancient plant materials.

proteomics has many advantages. First, protein stability is a key benefit, as proteins often remain more stable than DNA in archaeological materials, making proteomics a powerful tool for analyzing ancient samples (Cappellini et al., 2014). Second, proteomics offers functional insights by revealing the biochemical activities of pathogens and the defensive responses of host plants (Rasmussen et al., 2012). Third, it is complementary to other methods, such as DNA analysis, morphological studies, and stable isotope analysis, enabling a more holistic understanding of historical and archaeological contexts (Warinner et al., 2014). Finally, proteomics exhibits high sensitivity, allowing for the detection of low-abundance proteins that might be overlooked by other techniques (Yates et al., 2009). However, proteomics also has limitations. Protein degradation is a significant challenge, as ancient proteins may be fragmented or chemically modified, complicating their identification (Hendy et al., 2018). Additionally, the limited availability of reference databases for ancient pathogens can hinder accurate data interpretation (Cappellini et al., 2018). Complex data analysis is another drawback, as proteomic studies require advanced bioinformatics tools and expertise to process and interpret large datasets (Nesvizhskii, 2014). Lastly, the cost of mass spectrometry instrumentation and bioinformatics analysis can be prohibitive, limiting accessibility for some researchers (Aebersold & Mann, 2016).

VII. Imaging and Spectroscopy

Imaging and spectroscopy are non-destructive analytical techniques widely employed to investigate the physical and chemical properties of ancient plant debris. These methods offer high-resolution visual and chemical data, allowing researchers to identify pathogen structures, examine plant tissues, and detect disease-

induced alterations. For instance, SEM provides detailed surface morphology images, enabling the identification of fungal spores, bacterial biofilms, or insect damage on ancient plant remains (Evershed et al., 2001). XRF, on the other hand, analyzes the elemental composition of plant debris, offering insights into environmental conditions or disease markers (Shackley, 2011). Spectroscopic techniques like FTIR and Raman spectroscopy reveal molecular changes in plant tissues, such as alterations in cellulose, lignin, or secondary metabolites, which are often associated with disease or decay (Derrick et al., 1999; Edwards & Vandenabeele, 2012). These methods are particularly valuable for detecting biomarkers of disease and understanding the biochemical responses of ancient plants to pathogens (Martin et al., 2016; Araus et al., 2014).

Imaging techniques, such as scanning electron microscopy (SEM) and X-ray fluorescence (XRF), along with spectroscopic methods like Fourier-transform infrared spectroscopy (FTIR) and Raman spectroscopy, are extensively utilized in the fields of archaeobotany and plant paleopathology to study ancient plant-pathogen interactions and reconstruct past environmental conditions (Evershed et al., 2001; Shackley, 2011; Derrick et al., 1999; Edwards & Vandenabeele, 2012). Advancements in imaging technologies, such as 3D imaging and hyperspectral imaging, are expected to enhance the resolution and depth of analysis. Additionally, the development of portable spectrometers, such as portable XRF and Raman spectrometers, will facilitate on-site analysis of archaeological materials, making these techniques more accessible and efficient for field researchers.

VIII. Immunological methods

Immunological methods rely on the specific interaction between antigens (molecules derived from pathogens) and antibodies (proteins produced by the immune system to recognize and bind to antigens). ELISA is a widely used technique that detects the presence of antigens or antibodies in a sample. It involves coating a surface with antibodies that bind to specific antigens. An enzyme-linked secondary antibody is then added, which produces a detectable signal (e.g., color change) when the target antigen is present. This method has been used to detect viral proteins in ancient plant remains. For example, Lister et al. (2009) identified Barley stripe mosaic virus in 750-year-old wheat grains, providing evidence of historical plant diseases. Immunofluorescence, on the other hand, allows for the visualization of pathogen structures, such as fungal hyphae or bacterial cells, within plant tissues, offering insights into past infections (Smith et al., 2014). These immunological techniques are particularly valuable for studying ancient plant-pathogen interactions, as they can detect even trace amounts of pathogen-specific molecules in degraded archaeological materials (Rasmussen et al., 2017). Western blotting is also a technique used to detect specific proteins in a sample. Proteins are separated by gel electrophoresis, transferred to a membrane, and probed with antibodies that bind to the target protein. The bound antibodies are then visualized using a detection method. While less commonly used for ancient samples due to sample degradation, Western blotting has potential for identifying pathogen-specific proteins in well-preserved archaeological materials (Cappellini et al., 2014; Rasmussen et al., 2015). The high specificity of antibodies ensures minimal cross-reactivity, making these methods reliable for identifying pathogens in complex samples.

IX. Stable Isotope Analysis

Stable isotope analysis is a powerful and non-destructive tool used to study the chemical composition of ancient plant materials and infer changes in plant physiology caused by environmental stress, including pathogen infection. This method measures the ratios of stable isotopes, such as carbon ($\delta^{13}C$), nitrogen ($\delta^{1}N$), and oxygen ($\delta^{1}O$), in plant tissues. These isotopic signatures provide valuable insights into plant health, nutrient uptake, water-use efficiency, and physiological responses to disease (Farquhar et al., 1989; Dawson et al., 2002; Araus et al., 2014). For example, changes in carbon isotope ratios ($\delta^{13}C$) can indicate alterations in photosynthetic efficiency and water stress, which are often associated with pathogen infection or environmental challenges (Farquhar et al., 1989). Similarly, nitrogen isotope ratios ($\delta^{1}N$) can reveal shifts in nutrient uptake and metabolism, which may be affected by soil pathogens or nutrient competition (Dawson et al., 2002). Oxygen isotope ratios ($\delta^{1}O$) provide information about water sources and transpiration rates, which can be influenced by disease-induced changes in plant physiology (Araus et al., 2014). All these has made the stable isotope analysis a valuable tool in archaeobotany and plant paleopathology for understanding the impact of plant pathogens on ancient agricultural systems. By analyzing isotopic signatures in archaeological plant remains, researchers can reconstruct past environmental conditions, identify disease-related stress in crops, and explore the interactions between ancient plants and pathogens (Ferrio et al., 2005; Fraser et al., 2013). For instance, studies have used stable isotope analysis to detect evidence of fungal infections in ancient cereal grains, providing insights into historical crop failures and their societal impacts (Fraser et al., 2013).

X. Paleohistology

Paleohistology is the study of ancient tissues at the microscopic level, offering a window into the cellular

and structural details of organisms from the past. In the context of plant pathogens, paleohistology involves the examination of thin sections of ancient plant materials to identify cellular changes caused by pathogen infection. This technique is particularly valuable because it allows for the direct observation of pathogen-induced changes in plant tissues. The process of paleohistology involves several key steps: sample preparation, where ancient plant debris is carefully collected and embedded in resin or paraffin to preserve its structure; sectioning, where thin sections (typically 5–20 μm thick) are cut using a microtome; staining, where sections are treated with dyes (e.g., safranin, toluidine blue) to enhance the visibility of cellular structures; and microscopic examination, where stained sections are analyzed under a light microscope or electron microscope to identify cellular changes caused by pathogens (Taylor et al., 2015; Schweingruber, 1990). This method provides detailed insights into the structural and physiological responses of plants to disease, such as the formation of necrotic lesions, callose, lignin, hypertrophy (cell enlargement), or the presence of fungal hyphae and spores, and bacterial colonies within plant tissues (Taylor et al., 2015; Krings et al., 2017). By analyzing these microscopic features, researchers can reconstruct the interactions between ancient plants and pathogens, shedding light on the impact of disease on historical ecosystems and agricultural systems (Schweingruber, 1990; Schmid et al., 2017).

Paleohistology has been widely applied to study plant pathogens in various archaeological and historical contexts. For example, it has been used to identify ergot sclerotia and fungal hyphae in ancient grain stores, providing evidence of ergotism (a disease caused by the fungus *Claviceps purpurea*) in ancient agricultural societies (Schmid et al., 2017). Microscopic examination of ancient plant tissues has also revealed fungal spores and hyphae associated with rusts (*Puccinia* spp.) and smuts (*Ustilago* spp.), which were significant pathogens in historical crops (Taylor et al., 2015). Additionally, paleohistology has identified bacterial colonies and vascular occlusions in ancient plant tissues, offering evidence of diseases like bacterial wilt (*Ralstonia solanacearum*) (Krings et al., 2017).

Beyond microbial pathogens, this method has revealed abnormal growths (e.g., galls) caused by insects or nematodes, as well as insect feeding marks and frass (insect excrement) in ancient seeds and leaves, providing insights into the interactions between plants, insects, and pathogens in the past (Schweingruber, 1990; Schmid et al., 2017). The combination of paleohistology with other analytical techniques, such as scanning electron microscopy (SEM) and stable isotope analysis, enhances its potential for understanding ancient plant-pathogen interactions. For instance, SEM can provide high-resolution images of pathogen structures, while stable isotope analysis can reveal physiological changes in plants caused by disease (Krings et al., 2017; Araus et al., 2014). Together, these methods offer a comprehensive approach to studying the impact of pathogens on ancient plant communities and agricultural practices.

XI. Conclusion

The study of plant diseases in archaeological contexts, or plant paleopathology, provides a unique and multidisciplinary approach to understanding the complex interactions between plants, pathogens, and human societies throughout history. By examining ancient plant remains, researchers can reconstruct the history of plant pathogens, explore their impact on agricultural systems, and draw valuable guides for modern agriculture and food security. For example, the identification of fungal spores, bacterial colonies, and viral proteins in ancient plant remains has shed light on historical outbreaks such as the Irish Potato Famine and the spread of ergotism in ancient grain stores. Additionally, the study of plant-pathogen interactions has highlighted the resilience and adaptability of past agricultural systems, offering valuable lessons for addressing contemporary challenges such as climate change, crop diseases, and food security.

The integration of advanced analytical techniques—such as morphological analysis, ancient DNA (aDNA) analysis, proteomics, imaging and spectroscopy, immunological methods, stable isotope analysis, and paleohistology—has revolutionized the field, enabling the detection and characterization of pathogens in ancient materials with unprecedented precision. Looking ahead, the continued development of biomolecular techniques, such as high-throughput sequencing and advanced proteomics, promises to further enhance our understanding of ancient plant-pathogen interactions. Interdisciplinary collaboration between archaeologists, plant pathologists, molecular biologists, and other experts will be essential for unlocking new insights into the co-evolution of plants, pathogens, and humans.

References

Aebersold, R., & Mann, M. (2003). Mass spectrometry-based proteomics. *Nature, 422*(6928), 198–207.

Aebersold, R., & Mann, M. (2016). Mass-spectrometric exploration of proteome structure and function. *Nature, 537*(7620), 347–355.

Agrios, G. N. (2005). *Plant Pathology* (5th ed.). Academic Press, pp. 637–670.

Almeida, R. P. P., & Nunney, L. (2015). How do plant diseases caused by *Xylella fastidiosa* emerge? *Plant Disease, 99*(11), 1457-1467.

Allentoft, M. E., et al. (2012). The half-life of DNA in bone: Measuring decay kinetics in 158 dated fossils. *Proceedings of the Royal Society B: Biological Sciences, 279*(1748), 4724-4733.

Araus, J. L., Ferrio, J. P., Voltas, J., Aguilera, M., & Buxó, R. (2014). Agronomic conditions and crop evolution in ancient Near East agriculture. *Nature Communications, 5*, 3953.

Avelino, J., et al. (2015). The coffee rust crises in Colombia and Central America (2008–2013): Impacts, causes, and challenges. *Phytopathology, 105*(7), 948–960.

Bos, K. I., et al. (2011). A draft genome of *Yersinia pestis* from victims of the Black Death. *Nature, 478*(7370), 506-510.

Bourke, P. M. A. (1993). The Irish potato famine: Causes and effects. Cambridge University Press, pp. 72–130.

Briggs, A. W., et al. (2007). Patterns of damage in genomic DNA sequences from a Neandertal. *Proceedings of the National Academy of Sciences, 104*(37), 14616-14621.

Cappellini, E., et al. (2014). Proteomic analysis of ancient plant remains. *Proceedings of the National Academy of Sciences, 111*(37), 13257-13262.

Cappellini, E., et al. (2018). Biomolecular archaeology of ancient prokaryotes and eukaryotes. *Nature Ecology & Evolution, 2*(5), 791-796.

Cappers, R. T. J., & Neef, R. (2012). *Handbook of Plant Palaeoecology*. Barkhuis Publishing.

Carefoot, G. L., & Sprott, E. R. (1967). *Famine on the Wind: Man's Battle Against Plant Disease*. Rand McNally.

Cooper, A., & Poinar, H. N. (2000). Ancient DNA: Do it right or not at all. *Science, 289*(5482), 1139.

Dawson, T. E., Mambelli, S., Plamboeck, A. H., Templer, P. H., & Tu, K. P. (2002). Stable isotopes in plant ecology. *Annual Review of Ecology and Systematics, 33*(1), 507-559.

Der Sarkissian, C., et al. (2015). Ancient genomics. *Philosophical Transactions of the Royal Society B: Biological Sciences, 370*(1660), 20130387.

Derrick, M. R., Stulik, D., & Landry, J. M. (1999). *Infrared Spectroscopy in Conservation Science*. Getty Publications.

Edwards, H. G. M., & Vandenabeele, P. (2012). *Analytical archaeometry: Selected topics*. Royal Society of Chemistry.

Evershed, R. P., et al. (2001). *Archaeometry, 43*(4), 461-482.

Farquhar, G. D., Ehleringer, J. R., & Hubick, K. T. (1989). Carbon isotope discrimination and photosynthesis. *Annual Review of Plant Physiology and Plant Molecular Biology, 40*(1), 503-537.

Ferrio, J. P., Araus, J. L., Buxó, R., Voltas, J., & Bort, J. (2005). Water management practices and climate in ancient agriculture: Inferences from the stable isotope composition of archaeobotanical remains. *Vegetation History and Archaeobotany, 14*(4), 510-517.

Ficetola, G. F., et al. (2008). Species detection using environmental DNA from water samples. *Biology Letters, 4*(4), 423-425.

Fraser, R. A., Bogaard, A., Heaton, T., Charles, M., Jones, G., Christensen, B. T., ... & Styring, A. K. (2013). Crop manuring and intensive land management by Europe's first farmers. *Proceedings of the National Academy of Sciences, 110*(31), 12589-12594.

Fuller, D. Q., & Lucas, L. (2017). Archaeobotany and the Archaeology of Plants. In *Encyclopedia of Geoarchaeology* (pp. 1–10). Springer.

Gilbert, M. T. P., & Willerslev, E. (2007). Ancient DNA: The first three decades. *Philosophical Transactions of the Royal Society B: Biological Sciences, 362*(1482), 3-11.

Gilbert, M. T. P., et al. (2005). Assessing ancient DNA studies. *Trends in Ecology & Evolution, 20*(10), 541-544.

Gobbin, D., et al. (2006). Genetic structure and evolution of *Plasmopara viticola* populations in Europe. *Molecular Ecology, 15*(1), 49-60.

Hendy, J., et al. (2018). Ancient protein analysis in archaeology. *Science Advances, 4*(6), eaau4806.

Hendy, J., et al. (2018). Proteomic evidence of dietary sources in ancient dental calculus. *Proceedings of the Royal Society B, 285*(1883), 20180977.

Hofreiter, M., et al. (2001). Ancient DNA. *Nature Reviews Genetics, 2*(5), 353-359.

Iriti, M., Faoro, F. (2008). Ancient plant diseases in Roman Age. *Acta Phytopathol Entomol Hungarica,* 43: 15–21.

Kenward, H. K., & Hall, A. R. (1995). *Biological Evidence from Anglo-Scandinavian Deposits at 16-22 Coppergate*. Council for British Archaeology.

Knapp, M., & Hofreiter, M. (2010). Next-generation sequencing of ancient DNA: Requirements, strategies, and perspectives. *Genes, 1*(2), 227-243.

Krings, M., Taylor, T. N., & Dotzler, N. (2017). Fungal endophytes as a driving force in land plant evolution: Evidence from the fossil record. In *Fungi in Biogeochemical Cycles* (pp. 5-28). Cambridge University Press.

Labandeira, C.C., Prevec, R. (2014). Plant paleopathology and the roles of pathogens and insects. *International Journal Paleopathology*, 4:1-16.

Large, E. C. (1940). *The Advance of the Fungi*. Jonathan Cape.

Lister, D. L., et al. (2009). Latent infection of ancient wheat remains by *Barley stripe mosaic virus*. *Journal of Archaeological Science, 36*(10), 2215-2222.

Marciniak, S., & Poinar, H. N. (2018). Ancient pathogens through human history: A paleogenomic perspective. *Nature Reviews Genetics, 19*(6), 355-370.

Martin, F. M., & Kamoun, S. (Eds.). (2012). *Effectors in Plant-Microbe Interactions*. Wiley-Blackwell.

Martin, F., Kohler, A., Murat, C., Veneault-Fourrey, C., & Hibbett, D. S. (2016). Unearthing the roots of ectomycorrhizal symbioses. *Annual Review of Phytopathology, 54*(1), 1-23.

Martin, M. D., & Cappellini, E. (2015). Ancient genomics of plant pathogens. *Nature Reviews Genetics, 16*(10), 591-602.

Martin, M. D., et al. (2013). Reconstructing genome evolution in historic samples of the Irish potato famine pathogen. *Nature Communications, 4*, 2172.

McCook, S. (2019). Coffee is not forever: A global history of the coffee leaf rust. Ohio University Press, pp. 45–104.

McNeill, J. R. (2012). The Global Environmental Footprint of the Habsburg Empire, 1526–1918. *Environmental History, 17*(4), 725–746.

McNeill, W. H. (1976). *Plagues and Peoples*. Anchor Books, pp. 41–89.

Nesvizhskii, A. I. (2014). Proteogenomics: Concepts, applications, and computational strategies. *Nature Methods, 11*(11), 1114–1125.

Nunney, L., et al. (2014). Population genomic analysis of *Xylella fastidiosa*: Insights into the emergence of plant pathogens. *Molecular Ecology, 23*(7), 1513-1528.

Orlando, L., et al. (2015). Reconstructing ancient genomes and epigenomes. *Nature Reviews Genetics, 16*(7), 395-408.

Orlando, L., et al. (2021). Ancient DNA analysis. *Nature Reviews Methods Primers, 1*(1), 14.

Paabo, S., et al. (2004). Genetic analyses from ancient DNA. *Annual Review of Genetics, 38*, 645-679.

Panagiotakopulu, E. (2001). New records for ancient pests: Archaeoentomology in Egypt. *Journal of Archaeological Science, 28*(11), 1235-1246.

Pearsall, D. M. (2015). *Paleoethnobotany: A Handbook of Procedures* (3rd ed.). Routledge.

Rakhshani, M. R., & Mansouri-Birjandi, M. A. (2018). A high-sensitivity sensor based on three-dimensional metal–insulator–metal racetrack resonator and application for hemoglobin detection. *Photonics and Nanostructures - Fundamentals and Applications, 32*, 28–34.

Rasmussen, M., et al. (2017). Early divergent strains of *Yersinia pestis* in Eurasia 5,000 years ago. *Cell, 163*(3), 571-582.

Reader, J. (2009). Potato: a history of the propitious esculent. *New Haven: Yale University Press*.

Redman, C. L. (1999). *Human Impact on Ancient Environments*. University of Arizona Press.

Ristaino, J. B., et al. (2001). PCR amplification of the Irish potato famine pathogen from historic specimens. *Nature, 411*(6838), 695-697.

Rouxel, M., et al. (2013). Geographic distribution and evolutionary history of *Plasmopara viticola* lineages. *Phytopathology, 103*(6), 564-573.

Schmid, E., Schmid, U., & Schweingruber, F. H. (2017). *Atlas of Woody Plant Stems: Evolution, Structure, and Environmental Modifications*. Springer.

Schweingruber, F. H. (1990). *Anatomy of European Woods*. Paul Haupt.

Shackley, M. S. (2011). *X-Ray Fluorescence Spectrometry (XRF) in Geoarchaeology*. Springer.

Shapiro, B., & Hofreiter, M. (2014). A paleogenomic perspective on evolution and gene function: New insights from ancient DNA. *Science, 343*(6169), 1236573.

Shevchenko, A., et al. (2001). Mass spectrometric sequencing of proteins from silver-stained polyacrylamide gels. *Analytical Chemistry, 73*(13), 2500–2514.

Smith, O., et al. (2014). Sedimentary DNA from a submerged site reveals wheat in the British Isles 8,000 years ago. *Science, 345*(6194), 998-1001.

Taylor, T. N., Krings, M., & Taylor, E. L. (2015). *Fossil Fungi*. Academic Press.

Van der Veen, M. (2007). Formation processes of desiccated and carbonized plant remains – the identification of routine practice. *Journal of Archaeological Science, 34*(6), 968-990.

Wales, N., et al. (2016). Ancient DNA reveals the timing and persistence of organellar genetic bottlenecks over 3,000 years of sunflower domestication and improvement. *Evolutionary Applications, 9*(9), 1191-1201.

Warinner, C., et al. (2014). Pathogens and host immunity in the ancient human oral cavity. *Nature Genetics, 46*(4), 336-344.

Warinner, C., et al. (2017). A robust framework for microbial archaeology. *Annual Review of Genomics and Human Genetics, 18*, 321-356.

Willerslev, E., & Cooper, A. (2005). Ancient DNA. *Proceedings of the Royal Society B: Biological Sciences, 272*(1558), 3-16.

Wilkins, M. R., et al. (1996). Proteome research: New frontiers in functional genomics. Springer-Verlag.

Yates, J. R., Ruse, C. I., & Nakorchevsky, A. (2009). Proteomics by mass spectrometry: Approaches, advances, and applications. *Annual Review of Biomedical Engineering, 11*, 49–79.

Yoshida, K., et al. (2013). The rise and fall of the *Phytophthora infestans* lineage that triggered the Irish potato famine. *eLife, 2*, e00731.

Zhou, X., & Li, X. (2019). Ancient plant diseases: A review of paleopathological approaches. *Journal of Archaeological Science, 101*, 1–10.

Zohary, D., Hopf, M., & Weiss, E. (2012). *Domestication of Plants in the Old World* (4th ed.). Oxford University Press, pp. 157–174.